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RESPONSE UNDER C.F.R. § 1.116
EXPEDITED PROCEDURE
ART UNIT 1646

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Monty Krieger, Susan L. Acton, and Alan M. Pearson		
Serial No.:	08/765,108	Art Unit:	1646
Filed:	March 27, 1997	Examiner:	John D. Ulm
For:	*CLASS B1 AND C1 SCAVENGER RECEPTORS*		

Box AF
Assistant Commissioner for Patents
Washington, D.C. 20231

TRANSMITTAL OF THE EXECUTED DECLARATION

Sir:

Further to the Response to the Office Action mailed on February 10, 2003, enclosed is the executed declaration under 37 C.F.R. 1.132 of Monty Krieger. Please use the nine (9) exhibits ("Molecular Biology of the Cell", 3rd edition, Garland Publishing, Inc. New York (1994) (Exhibit 1), copied excerpts from "Current Protocols in Molecular Biology" (Exhibit 2), 3 articles containing copied pages from 3 molecular biology textbooks: Molecular Biology of the Cell, 3rd edition, Garland Publishing, Inc. New York (1994) (Exhibit 3); Recombinant DNA, 2nd edition, Scientific American Books, New York (1992) (Exhibit 4); and Genes V, Oxford University Press, Oxford (1994)(Exhibit 5), and 3 Medline Abstracts: Hirano *et al.*, Proc Natl Acad Sci, 80:46-50 (Exhibit 6); Campbell and Porter, Proc Natl Acad Sci, 80:4464-8 (Exhibit

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7); Degrave *et al.*, Mol Biol Rep, 11:57-61 (Exhibit 8) and one copy of 35 lab notebook pages (Exhibit 9)), submitted with the response filed February, 2003.

Respectfully submitted,

Patrea L. Pabst
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Date: February 14, 2003

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Certificate of Facsimile Transmission

I hereby certify that this document, and any documents referred to as attached therein are being facsimile transmitted on this date, February 14, 2003, to the Commissioner for Patents, U.S. Patent and Trademark Office, Washington, DC 20231.

Aisha Wyatt

Date: February 14, 2000

ATL1 #564573 v1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Monty Krieger, Susan L. Acton, and Alan M. Pearson		
Serial No.:	08/765,108	Art Unit:	1646
Filed:	March 27, 1997	Examiner:	John Ulm
For:	CLASS BI AND CI SCAVENGER RECEPTORS		

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. §1.132

Sir:

We, Monty Krieger and Susan L. Acton, hereby declare that:

1. We are the co-inventors of the claimed subject matter in the above-identified patent application. We affirm the statements made in our declaration under 37 C.F.R. 1.131 executed April 24 and 25, 1996 in the parent application U.S.S.N. 08/265,428 filed June 23, 1994.

2. A cDNA is different from a genomic DNA but still retains the sequences encoded by the exons of the genomic DNA (the gene). It is defined as a "DNA molecule made as a copy of mRNA and therefore lacking the introns that are present in genomic DNA. Used to determine the amino acid sequence of a protein by DNA sequence or to make the protein in large quantities by cloning followed by expression.": from Molecular Biology of the Cell, 3rd edition, Garland Publishing, Inc. New York (1994) (Exhibit 1) The

cDNA molecule is reverse transcribed from the mRNA molecule that encodes the protein, in this case, SR-BI.

3. The field of cloning at the time of filing, June 23, 1994, was quite developed. Protocols were established in the late 1970's and early 1980's for generating recombinant DNA libraries which still exist as current protocols in the art. (Current Protocols in Molecular Biology; Exhibit 2) Genomic libraries were created by partial digestion of genomic DNA followed by size fractionation to remove fragments too small or too large to clone. The fragments are ligated into λ phage or cosmid vectors and transformed into *E. coli*. After plating, each colony on the bacterial substrate carries a genomic fragment and can be screened using a DNA probe. Many of these libraries were made commercially available, or traded among researchers in the early 1990s.

Methods for screening libraries with cDNA probes were also routine in the art at this time. As also described in Current Protocols in Molecular Biology (page 6.3.1; Exhibit 2), colonies can be transferred to nitrocellulose filters and probed with a cDNA probe radiolabeled with ^{32}P. Probe labeling kits were available at this time. The probe can be end-labeled or labeled by incorporation of a radiolabeled nucleotide in the sequence. This procedure was routine at the time of filing and allowed for a rapid, effective screening procedure that allowed for detection a single clone containing the genomic fragment in a population of millions of clones. The filters are hybridized under standard hybridization conditions to allow the probe to bind to complementary sequences on the filter. The temperature and salt concentration in the hybridization buffer can be adjusted to modify

the stringency of the hybridization. Protocols and parameters for probe hybridization are referenced from Chapter 11 of the well known laboratory manual of Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, New York (1990) incorporated by reference on page 40 of our specification. High stringency conditions will only allow exactly complementary sequences to bind while lower stringency hybridization conditions will allow for a probe to bind similar, but not identical, sequences. Excess unbound probe is washed off in a series of washes after the incubation with probe. The resulting filter is exposed to X-ray film and "autoradiography" demonstrates which clone contains the fragment complementary to the labeled probe. The clone of interest can be used to inoculate large-scale plasmid preparations for the isolation of the genomic fragment corresponding to the cDNA probe. Plasmid isolation kits were commercially available in the 1990s by QIAGEN® based on well-known principles and routine protocols known in the art. Further, these techniques have become standard material in molecular and cellular biology textbooks as demonstrated by excerpts from Molecular Biology of the Cell, 3rd edition, Garland Publishing, Inc. New York (1994), Recombinant DNA, 2nd edition, Scientific American Books, New York (1992), and Genes V, Oxford University Press, Oxford (1994) (Exhibits 3-5 respectively)

It was routine to screen genomic libraries using cDNA hybridization probes as much as a decade before this filing date within and between species. Examples such as Hirano *et al. Pro Natl Acad Sci* 1983 80:46-50, Campbell and Porter *Proc Natl Acad Sci* 1983 80:4464-8, and Degrave *et al. Mol Biol Rep* 1986 11:57-61(Exhibits 6-8 respectively)

demonstrate isolation of genes from genomic libraries by screening with a cDNA probe. The hybridization conditions such as temperature and salt concentration can be adjusted to decrease the stringency of the probe hybridization. A lower stringency condition would allow for a probe to bind sequences that were very similar but not identical to the cDNA probe. This would be ideal for screening genomic libraries from different species for homologous sequences. The article by Degrave *et al.* (Exhibit 8) specifically describes successful screening of a mouse genomic library with a human cDNA probe using non-stringent hybridization conditions to isolate the gene for interleukin-2.

4. One of skill in that art would have known in 1994 how to make, or obtain a human genomic library and synthesize a radiolabeled cDNA probe with routine experimentation. Probing nitrocellulose filters with the radiolabeled cDNA probe was already established and widely used in the art. These techniques were established and used routinely for at least 10 years prior to filing of this application.

The present specification provides a clear and enabling disclosure of these methods. The general methods to isolate genomic DNA sequences by probing a library with a nucleic acid probe are described on page 10, lines 2-20. Detailed description of using nucleic acid probes is described on page 25, lines 10-20. The paragraph bridging pages 34 and 35 describes methods for making a genomic library. Methods to screen patient samples using hybridization assays are described on page 40, lines 6-16. The details of nucleic acid hybridization and stringency of reaction are described on page 40, line 18 to

page 41, line 19. The genomic DNA can be described and defined by the cDNA since the protein encoding sequences are the same.

5. In the present patent application, we demonstrate the isolation of a cDNA molecule for the hamster SR-BI isolated from a variant chinese hamster ovary cell line (Var-261) cDNA expression library. We discovered that this variant CHO cell line expressed an apparently novel polyanion binding scavenger receptor, that did not hybridize to a probe for the hamster type I and II class A receptors. A cDNA expression library was made and transfected into COS cells and screened for uptake of fluorescent AcLDL. A receptor positive clone was isolated and found to contain the plasmid containing hamster SR-BI cDNA. It was noted that SR-BI was not expressed in very high levels in Var-261 cells, and therefore was not the receptor responsible for the observed polyanion binding activity, it was determined to be a novel scavenger receptor protein by analysis of its function (i.e. binding activity) and sequence.

The hamster SR-BI cDNA was characterized and found to be homologous with members of the CD36 family of membrane proteins in human, rat and drosophila. After transient transfection in COS cells, the hamster SR-BI plasmid conferred high affinity ^{125}I-AcLDL binding on the COS cells.

The tissue distribution of SR-BI was determined in RNA samples from murine kidney, liver, brain, testis, fat, diaphragm, heart, lung, spleen or fibroblasts by Northern Blot using a 600bp fragment of the coding region of hamster SR-BI. SR-BI was highly expressed in fat and to lower degrees in liver and lung.

6. As demonstrated by copies of the laboratory notebook pages of Dr. Susan L. Acton (Exhibit 9), we were able to use the hamster cDNA sequence to generate a radiolabeled probe and screen a mouse 3T3-LI adipocyte library for the corresponding mouse SR-BI sequence using established protocols in the art. The probe corresponded to a 500-600 bp BamHI fragment (5') of hamster SR-BI in pcDNAI labeled with [α^{32}P]dCTP incorporated in the sequence. Hybridization was performed in 500mM phosphate buffer at 50°C for the first round of selection. Phosphate buffer concentration was lowered to 300mM for the second and third rounds of hybridization at 50°C to isolate mouse SR-BI. This shows that one of ordinary skill in the art could have isolated an SR-BI clone from a different mammalian species using the hamster cDNA as a probe under less stringent hybridization conditions.

7. We are familiar with the publication by Calvo, et al., *J Biol Chem* 268(25): 18929-18935, (September 5, 1993). Calvo et al., isolated a human gene encoding a protein of unknown function based on its homology to CD36 and LIMPII. After we determined the structure and a functional activity of the hamster class B1 scavenger receptor protein, described and claimed in the above-identified patent application, it was apparent to us that the gene isolated by Calvo, et al. encodes the human homologue of the hamster class B1 scavenger receptor protein.

8. Calvo *et al.* reports a nucleic acid and predicted protein sequence for CLA-1. CLA-1 is now known to be the human homologue of SR-BI. Calvo did not express the full length protein nor did they characterize it. Therefore, they did not know what the

protein was nor how to use it. The previous declaration executed April 24 and 25, 1996 demonstrated that we had conceived and reduced to practice in our laboratory at the Massachusetts Institute of Technology, Cambridge, MA, an isolated cDNA encoding scavenger receptor protein type BI, prior to September 5, 1993. Screening a human genomic library with the hamster cDNA as a probe under low stringency hybridization conditions would have identified the human homologue for SR-BI (i.e. CLA-1) in exactly the same manner that was routine in the art a decade earlier as demonstrated by Degrave *et al.*

9. Since the publication of Calvo in 1993, there was no reason for us to isolate the human homologue using the hamster cDNA as a probe. We were aware that the sequence in Calvo represented the human homologue of SR-BI which could routinely be isolated using the techniques we employed to isolate the mouse sequence. There was no reason for us to pursue the human SR-BI homologue because it was clear that if we probed a human genomic library, we would have obtained a sequence identical or substantially identical to the sequence in the GenBank database submitted by Calvo *et al.*

10. As persons of at least ordinary skill in the field of molecular biology, we believe that a person of skill in the art before June 23, 1994 would have been able to construct or obtain a suitable genomic library publicly available, synthesize a labeled cDNA probe using the cDNA disclosed in SEQ ID NO:3 of the present application and screen for the genomic homologues of SR-BI using protocols and guidance established in the art, and obtain the genomic DNA encoding SR-BI with only routine experimentation.

U.S.S.N. Monty Krieger and Susan L. Acton
Filed: March 27, 1997
CLASS BI and CI SCAVENGER RECEPTORS

11. We declare that all statements made herein of our own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further, that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 2/11/03 Dr. Monty Krieger

Date: _____ Dr. Susan L. Acton

ATL1 #559254 v1